



100

09 MAR 2003



10/527308  
PCT/GB 2003/002437



INVESTOR IN PEOPLE

The Patent Office

Concept House

Cardiff Road

Newport

South Wales

NP10 8QQ

REC'D 14 JUL 2003

WIPO

PCT

**PRIORITY  
DOCUMENT**

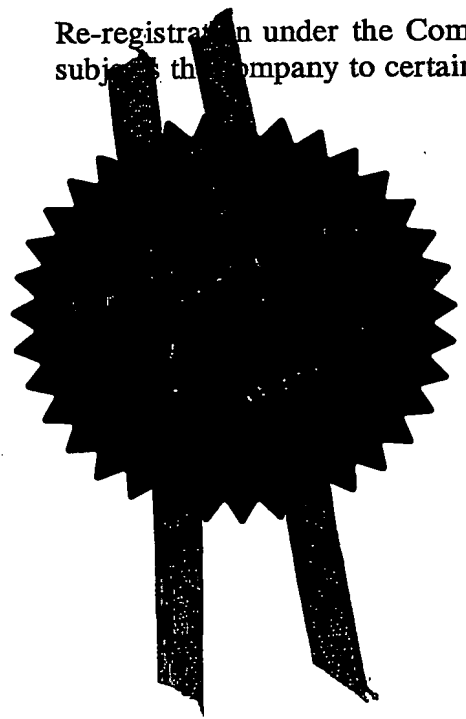
SUBMITTED OR TRANSMITTED IN  
COMPLIANCE WITH RULE 17.1(a) OR (b)

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

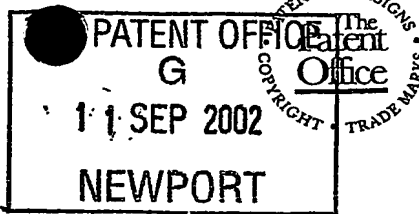


Signed

*P. Mahoney*

Dated

25 June 2003



11SEP02 E747347-1 D02819  
P01/7700 0.00-0221014.4

The Patent Office

Cardiff Road  
Newport  
South Wales  
NP10 8QQ

# Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1. Your reference

C1385.00/M

2. Patent application number

(The Patent Office will fill in this part)

0221014.4

11 SEP 2002

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Medical Research Council  
20 Park Crescent  
London  
W1B 1AL

Patents ADP number (if you know it)

596007001

If the applicant is a corporate body, give the country/state of its incorporation

England and Wales

4. Title of the invention

Spectral Discrimination Apparatus and Method

5. Name of your agent (if you have one)

KEITH W NASH & CO

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

90-92 Regent Street  
Cambridge  
CB2 1DP

Patents ADP number (if you know it)

1206001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number  
(if you know it)

Date of filing  
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing  
(day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

Yes

- a) any applicant named in part 3 is not an inventor, or
  - b) there is an inventor who is not named as an applicant, or
  - c) any named applicant is a corporate body.
- See note (d))

**Patents Form 1/77**

9. Enter the number of sheets for any of the following items you are filing with this form.  
Do not count copies of the same document

Continuation sheets of this form

Description 11

Claim(s)

Abstract

Drawing(s)

4 + 4 

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents


Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination and search (*Patents Form 9/77*)

Request for substantive examination (*Patents Form 10/77*)

Any other documents  
(please specify)

11. I/We request the grant of a patent on the basis of this application.

Signature   
KEITH W NASH & CO

Date  
10/9/2002

12. Name and daytime telephone number of person to contact in the United Kingdom

**Warning**

*After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.*

**Notes**

- a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.
- b) Write your answers in capital letters using black ink or you may type them.
- c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.
- e) Once you have filled in the form you must remember to sign and date it.
- f) For details of the fee and ways to pay please contact the Patent Office.

C1385.00M

**TITLE: SPECTRAL DISCRIMINATION APPARATUS AND METHOD**

**Field of the Invention**

This invention relates to spectral discrimination apparatus for use in scanning optical microscopes and to a method of use of such apparatus.

**Background to the Invention**

Several types of scanning optical microscope are now used. These include the confocal microscope and the multiphoton-excitation fluorescence microscope. In many such microscopes the illumination is confined to a spot of light which is scanned over the specimen, or alternatively the specimen is scanned relative to a stationary spot. The invention described here is applicable to all those forms of scanning optical microscope in which only a single spot is scanned.

A part of the established art of optical microscopy is the use of a series of lenses or other focussing means by which the specimen is focussed into a so-called intermediate image plane. This plane is one of a family of optically conjugate planes: as well as the aforementioned intermediate image plane the family includes the plane of focus within the specimen and the plane of the illumination iris and, in the case of systems with cameras, the plane of the photosensitive surface. This family of conjugate planes is known as the family of *image planes*. It is taught in elementary textbooks of microscopy that the aforementioned image planes alternate along the axis of a microscope with so-called *aperture planes*, in which the physical apertures of the lenses, or their back focal planes, are contained or imaged. Thus the family of aperture planes contains the condenser iris, the back focal plane of the objective lens and the Ramsden disc, (a small disc-shaped image of the back focal plane of the objective lens situated generally above the eyepiece and conjugate with the condenser iris). The terms "image plane" and "aperture plant" are

introduced here because they are useful in understanding both the prior art and the present invention.

White, in US Patent 5 032 720, taught the use of a variable iris, controlling the passage of light to the detector in a confocal microscope. The iris is placed in an image plane such that the iris is optically conjugate with the focussed spot of light in the specimen. The iris diaphragm is, in the above terminology, an image plane stop. This image plane stop has the function of blocking light from regions of the specimen that lie outside the focussed spot. With such a stop, the scanning microscope functions as a confocal microscope. White also taught the division of the emitted light coming from the specimen in a confocal microscope into more than one beam according to wavelength, with this division being effected by chromatic reflectors. This design has proved to have many applications, chiefly in the simultaneous imaging of a plurality of fluorescent stains of different fluorescence emission colours in the same specimen.

Brakenhoff, in a diagram published on page 189 of Confocal Microscopy, edited by T. Wilson, Academic Press 1990, showed how a spectrometer could be used in conjunction with a scanning confocal microscope as a substitute for the chromatic reflectors of White. The advantage of this is that the wavelength of light passing to the detector can be selected from a continuous range. It is obvious to those skilled in the art of optics that the entry aperture to the spectrometer must be placed in an aperture plane as defined above. Only in this way can the spectrometric discrimination of colours in the emitted light be applied equally to all points in the image plane.

To the knowledge of the applicants, the closest prior art to the current invention is probably that of Engelhardt (US Patent 5,886,784) in which a spectrometer is described "of a form suitable for confocal fluorescence microscopy". This spectrometer is shown in Figure 1 of the accompanying drawings as Prior Art to the current invention. Figure 1 is derived from a combination of Figures 1 and 3 of Engelhardt's US Patent.

With reference to Figure 1 of the accompanying drawings, a confocal microscope 1 comprises a laser 2 which emits a beam of light 3 which is reflected by a reflector 4 and so passes through a filter 5 and a lens 6 which focusses the beam on an aperture or diaphragm 7. The beam passes thence to a chromatic beamsplitter 8 where a part of the beam is reflected through a lens 9 on to a scanning mirror 10. From the scanning mirror 10, the beam passes into an eyepiece lens 11 and an objective lens 12, being focussed by the objective lens on to a specimen 13. Light emitted from the specimen 13 passes back through the apparatus, retracing its original path, to the chromatic beamsplitter 8, where a part of the beam is focussed by lens 9 along path 14 on to a second aperture 15. Engelhardt referred to this microscope as a confocal microscope and, for this to be true, the apertures in the diaphragms 7 and 15 must each be confocal with the spot of light on the specimen 13 and from the discussion above it is clear that both must lie in conjugate image planes.

Engelhardt's invention is shown in the parts of Figure 1 that lie to the left of the diaphragm 15. It consists of a spectrometer which takes the form of a dispersive optical component such as a prism or grating, into which the beam of light (which has already passed through the confocal aperture at 15) passes and is dispersed into a fan of differently-coloured beams which are separated by a mask 17 and a reflector 18 into portions, one portion passing to detector 19 and another portion corresponding to a different range of wavelengths passing to a different detector at 22. Numerals 20 and 21 in Figure 1 indicate opaque screens which can be translated linearly to block more or less of the spectrally-dispersed beam passing to detector 22. Although Engelhardt is silent on this point, it is obvious to those skilled in the art that for any and all wavelengths the masks must lie in or close to an aperture plane or its equivalent and certainly not in an image plane of the microscope. If this design principle were not followed, the spectral separation would be applied to some parts of the microscope image and differently to others. Also, enlargement of the confocal aperture would have a deleterious effect on the spectral resolution.

Engelhardt's invention works satisfactorily, but suffers from the following disadvantages. If a series of masks and baffles are used, as specified in his patent, they cannot all lie in the same aperture plane as required for ideal performance. Also, if, as he taught, the slits in the plural masks are variable both in width and position, a large number of independent linear actuators are needed, which are slow and expensive and introduce complexities of control and interpretation. The latter are particularly severe when the dispersive element is a glass prism, as in Engelhardt's preferred embodiment, in which case the dispersion angle is anomalous, obeying no simple physical law and characteristic of the glass type. This requires the application of computed correction to each motor position to compensate for anomalous dispersion. Finally, the use of plural detectors, though advantageous for the simultaneous detection of light of different wavelengths, militates against the production of a cheap or compact instrument.

The present invention aims to overcome these difficulties. It is applicable to all point-scanning confocal microscopes but works best with those containing a telescope in the emission path. Such a telescope is a well-known part of the art of confocal microscopy.

### **Summary of the Invention**

According to one aspect of the invention there is provided spectral discrimination apparatus for use in a scanning optical microscope, the spectral discrimination apparatus comprising dispersive means and frequency selective means including a rotatable member or members given a form which, on rotation of the member or members, controls the frequency of light transmitted by the apparatus. The apparatus preferably includes a detector for receiving light from the frequency selective means.

The (or each) rotatable member is preferably positioned at an aperture plane after the dispersive means, although this is not essential if the rotatable member has its outer periphery shaped to provide a cam surface engaged by a cam follower movement of which controls the frequency of light transmitted by the apparatus.

Where a single rotatable member acts selectively to block or transmit light, the member is formed or shaped to provide the required blocking or transmission of light, and this may be achieved by furnishing the rotatable member with opaque and transmissive regions, with one or more light-transmitting apertures in an otherwise opaque rotatable member, or by shaping the outer periphery of the rotatable member to control the passage of light past the rotatable member.

Where a pair of rotatable members are employed, they may be rotated about the same axis, or about spaced parallel axes, the members cooperating together to provide the required light blocking and transmission, for example by the outer peripheries of the members being shaped to define a light-transmitting slot between them.

The or each rotatable member may be in the form of a disc-like member and the or each member can be continuously rotated (for example at high speed to facilitate switching between lines in raster scanning) or rotated in a step-wise fashion between indexed positions. The rotation may cause a particular waveband or wavebands of light to be transmitted and this waveband may vary with angular rotation or may be invariable.

According to another aspect of the invention there is provided a method of spectral discrimination in a scanning optical microscope, comprising dispersing the light and passing the light through frequency selective means in which the form of a rotating member or members controls the frequency of transmitted light.

In the preferred embodiment of the invention, the microscope is a confocal microscope equipped in its emission light path (corresponding to 14 in Figure 1) with a telescope. The telescope acts on the light coming from the specimen in such a way as to produce an aperture plane conjugate with the back focal plane of the objective lens and containing an image of the back focal plane of the objective much reduced in size. This image, which corresponds to an exit pupil of the telescope, serves as the entrance pupil for a spectrometer. The spectrometer receives light emanating from this image and disperses it according to wavelength by passage of the light through a prism, a diffraction grating or



other dispersive means. Means (such as lenses) are also provided by which the light of each wavelength is brought to a focus. In the case that white light, consisting of a continuous series of wavelengths, enters the dispersing means, a rainbow-like streak is formed, containing an infinite series of images of the exit pupil of the telescope. The streak lies in an aperture plane conjugate with the other aperture planes in the microscope.

The frequency selection means, consisting in the simplest embodiment of a thin perforated disc, or an opaque disc with transparent regions, or a number of such discs, is positioned after the dispersive element and in the aperture plane containing the streak in such a way that the perforations control the admission of light of different wavelengths to the detector. Such discs can be produced very cheaply and controlled by a single cheap rotary motor or a small number of such motors, which can effect a switch of wavelength much more rapidly than the linear actuators of Engelhardt. The rotation of the disc to definite angular positions allows the wavelength of detection to be varied in steps. The disc can also be spun continuously to achieve high speeds of switching or subjected to an angular rotary vibration, so alternating rapidly between positions or scanning through a series of such positions.

The position, shape and size of the perforations in the disc are computed to allow correction for anomalous dispersion effects in glass prisms or to compensate for variation in detector sensitivity with wavelength. Perforations or other markers are optionally provided on the disc or discs or on devices mounted on the same axle or moving in coordination with the discs to allow the disc position to be determined electronically. Similar perforations or markers are optionally provided to serve to switch the incident light from a laser or lasers or other light sources in the confocal microscope.

### **Brief Description of the Drawings**

The invention will now be further described, by way of example, with reference to the accompanying drawings, in which:

Figure 1, already described, is illustrative of the prior art,

Figure 2 shows a preferred embodiment of spectral discrimination apparatus according to the invention, used in a confocal microscope, and

Figures 3 and 4 show various rotatable members of the spectral discrimination apparatus of Figure 2.

### Detailed Description

Referring to Figure 2, the spectral discrimination apparatus (shown by components referenced 31 to 37) is shown in a confocal microscope 1 having scanning components 2 to 13 corresponding to similarly numbered components in Figure 1. In Figure 2, light is shown entering a Keplerian telescope, consisting of lenses 23 and 24. The light entering lens 23 is equivalent to the light beam 14 of Figure 1: it consists of the light emitted by the specimen 13 in the scanning microscope 1. The telescope produces a focussed image of the back focal plane of the objective lens of the scanning microscope in a plane 25 which is, according to standard optical terminology, an aperture plane. The image of the back focal plane thus formed by the telescope is greatly reduced in size relative to the actual aperture size in the objective lens. Light beam 26 passes from this reduced-size image to a diaphragm 28, being deflected for convenience by a reflector 27. The diaphragm 28 is situated in an image plane where it can be closed down to provide a confocal aperture if the microscope is required to function as a confocal one. A portion of the light passes through the diaphragm 28 and enters a positive lens 29, which is separated from the plane 25 by its focal length. In accordance with the rules of geometrical optics, the beam 26 which spreads from the small image in the aperture plane 25 is rendered substantially parallel by the lens 29 and the light passes as a parallel beam 30 into a prism 31 or other dispersive means. The light of each individual wavelength continues as a parallel beam but at an angle to similar beams of other wavelengths. In Figure 2, light of three different wavelengths is shown passing into a positive lens 32 which focusses the parallel beams to respective foci at 33, 34 and 35. At each focus there is an image of the

image in aperture plane 25. The images of these and other wavelengths lie in a relayed aperture plane in which is placed a selector disc 36 rotatable about a central axis defined by an axle 37. The selector disc 36 has opaque and transparent regions and by rotation on the axle 37 light of different wavelengths, corresponding to different radial distances on the disc 36, may be allowed to pass through the disc, or be blocked. The light that is allowed to pass through the disc 36 passes to a detector 39, being optionally concentrated by lens 38.

Three different embodiments of the selector disc 36 are shown in Figure 3, where the opaque regions of the disc are shown by cross-hatching. Disc 40 is shown with 13 rectangular holes, but the number is arbitrary. The disc 40 is shown overlapping an elongated rectangular region 41, corresponding to the streak of dispersed images such as 33, 34 and 35 of Figure 2. With the disc 40 in the position shown relative to the streak of illumination at 41, only wavelengths in one short band are allowed to pass to the detector 39, via the slot 48. By rotation of the disc about the central circle, which indicates the axis of rotation defined by the axle 37, different slots are brought into the illuminated field. As is indicated in the drawing of disc 40, the slots can be positioned on a spiral to allow a succession of ascending or descending wavelengths to be admitted for detection. The radial extent of each slot determines the width of the optical passband. The precise shape of the spiral is optionally chosen so that equal angular shifts of the disc give equal shifts of the centre of the passband in terms of wavelength. This allows the disc to compensate for the non-linear dispersion of the glass of the prism 31. Alternatively, the slot width in the radial direction is chosen to compensate for variation in spectral sensitivity of the detector according to wavelength. As shown at 49 on disc 40, more than one slot is optionally placed on a single radial line, to allow detection in two different wavebands. The thin bridge of opaque material between the two slots at 49 provides for blockage of one very restricted range of wavelengths, such as that of a laser, which is of particular advantage in fluorescence and other modes of microscopy in which an intense laser source is used, but the emitted signal at a nearby wavelength is weak.

Disc 42 is a different embodiment, in which a continuous spiral slot 50 is formed in the disc. This allows the detection waveband to be scanned continuously through the spectrum as the disc is rotated and the transmission window moves across the dispersed light field 43.

Components 44 and 45 shown in Figure 3 are combined on the same axis to provide a compound disc 46 with a spiral slot. In this, different embodiment, the relative rotation of parts 44 and 45 is used to vary the slot width, thus changing the passband selected from the light at 47, while the rotation of the compound disc 46 changes the position of the centre wavelength of detection within the spectrum.

Figure 4 illustrates further embodiments of the selector disc. Spiral discs 51 and 53 can be rotated around different, parallel but spatially displaced axes, and both discs have shaped outer peripheries to control the spectrally-dispersed streak of light at 52. The separation of axes simplifies the motor drive of the discs relative to the disc 46. Reference numerals 54, 55 and 56 show a similar arrangement but with outer peripheries of spiral forms of opposite sense.

Numerals 57 and 58 show a disc and light field respectively, in which the profile of the disc edge has two spiral forms, and 59 and 60 a similar arrangement but with four spirals combined in one disc. Discs 57 and 59 could be used singly or in combination with other discs as in 46, 51 and 53 to provide more rapid scanning through the spectrum of wavelengths for a given motor speed.

Also included in the invention are discs, or elements mechanically linked to the discs, which bear slots or markers (including electronic sensors such as Hall effect sensors) with functions other than the selection of detected wavebands. These include simple perforations to identify a home position of the disc (e.g. by means of a slotted opto-switch), or encoder regions to allow an unambiguous readout of disc position or slots or markers allowing direct shuttering or indirect switching of illumination for the microscope. For example, the same disc that controls the detection wavelength may, according to the

invention, switch to an appropriate laser from a choice of several lasers, simultaneously with switching detection waveband.

Also included are discs in which the transmissive regions have optical filter properties, either incorporated into the material of the disc or attached thereto and rotating with the disc.

The invention also includes forms in which the spectral region is defined not directly by a disc, but by a cam-follower which follows the profile of a rotating cam and is thereby translated in such a way as to block a variable portion of the spectrally dispersed light.

Also included in the invention are a number of modes of operation. These include rotational movements of the disc or discs to successive indexed positions, i.e. the successive presentation of different selector disc positions by rotational steps of fixed angular size, and the continuous rotation of the selector disc or discs to facilitate more rapid switching of detection waveband, for example switching between lines in a raster scanning system. It is also envisaged that the disc or discs may be rotated, either continuously or in distinct angular increments, in an angular oscillatory fashion, for example, to alternate repeatedly between two or more detection wavebands. In a favoured mode of operation, the disc embodiments 46, 51, 54, 57 and 59 are rotated continuously while monochromatic light, such as from a laser, is reflected into the spectrometric system. As the disc is rotated, the point of breakthrough of this light of known wavelength is detected and from this, the wavelength scale of the instrument can be calibrated automatically.

By this means, each disc of the compound embodiments can be calibrated independently.

Also included in the invention are microscope systems in which plural detectors are used, placed in the light path subsequent to the selector disc. This includes systems in which other means of chromatic separation are used in combination with the selector disc, for example means of chromatic separation can be placed before or after the selector disc, or

even on the selector disc by using colour filters fixed or cemented to the surface of the disc.

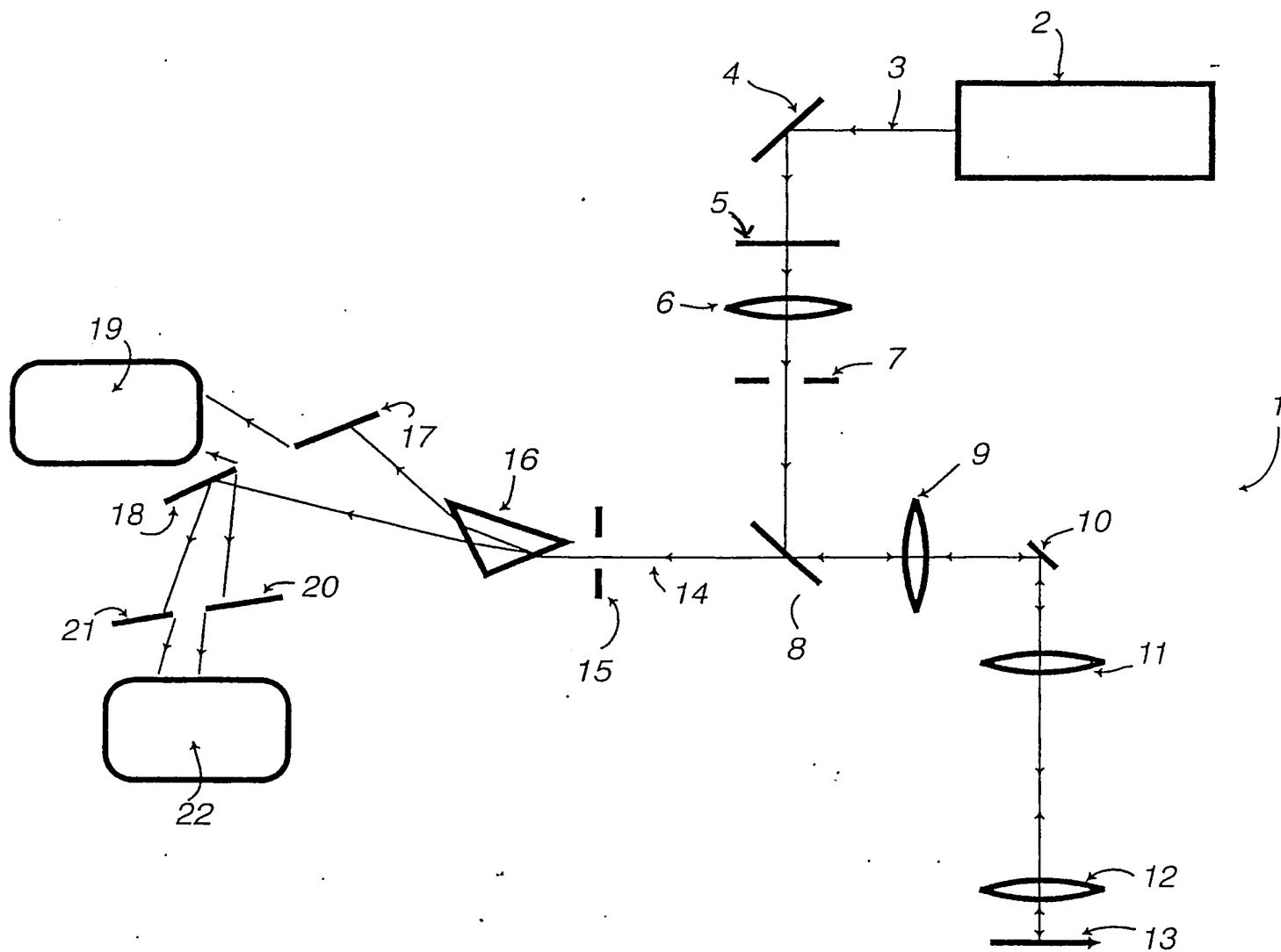


Figure 1 : Prior Art

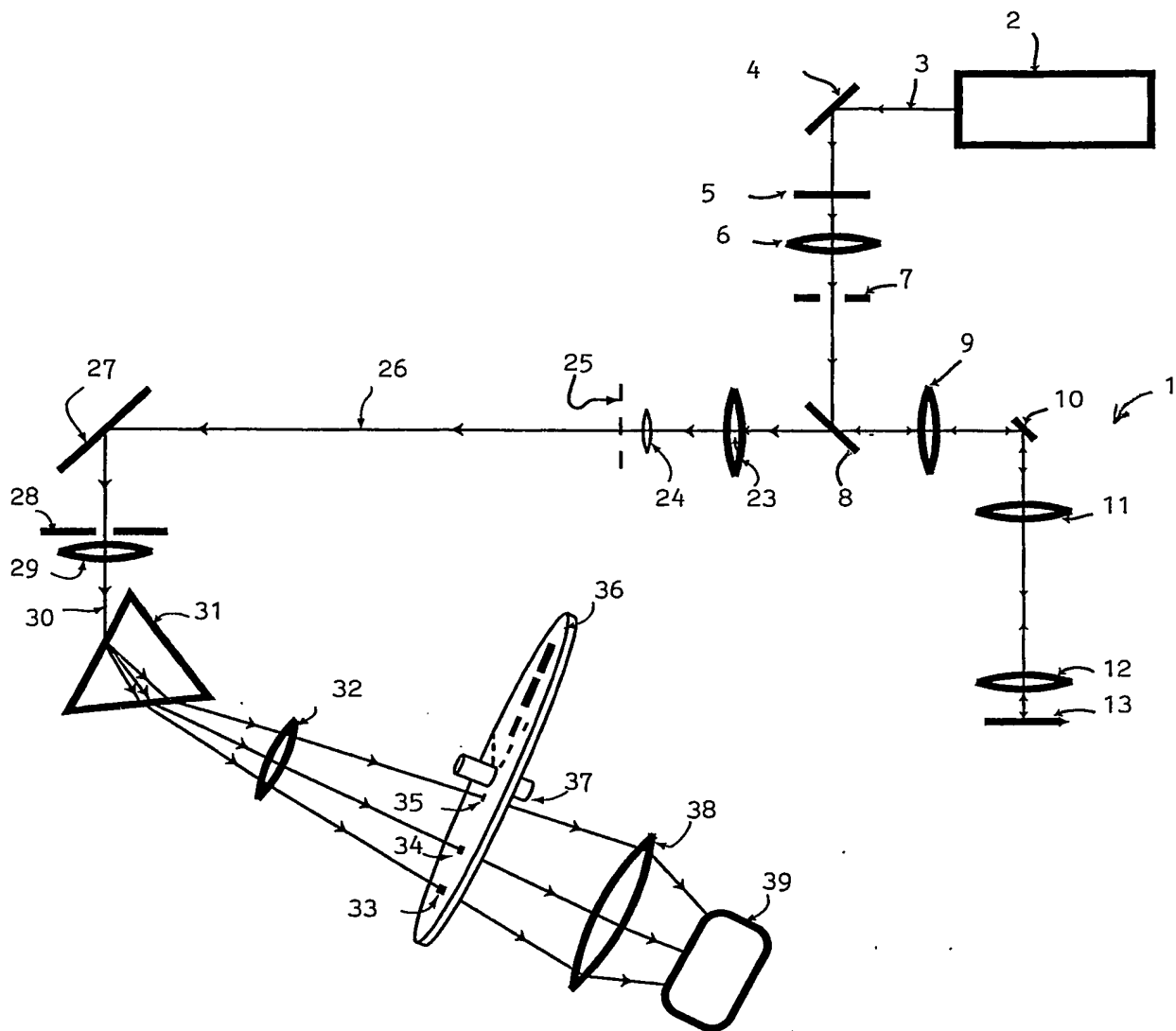


Figure 2



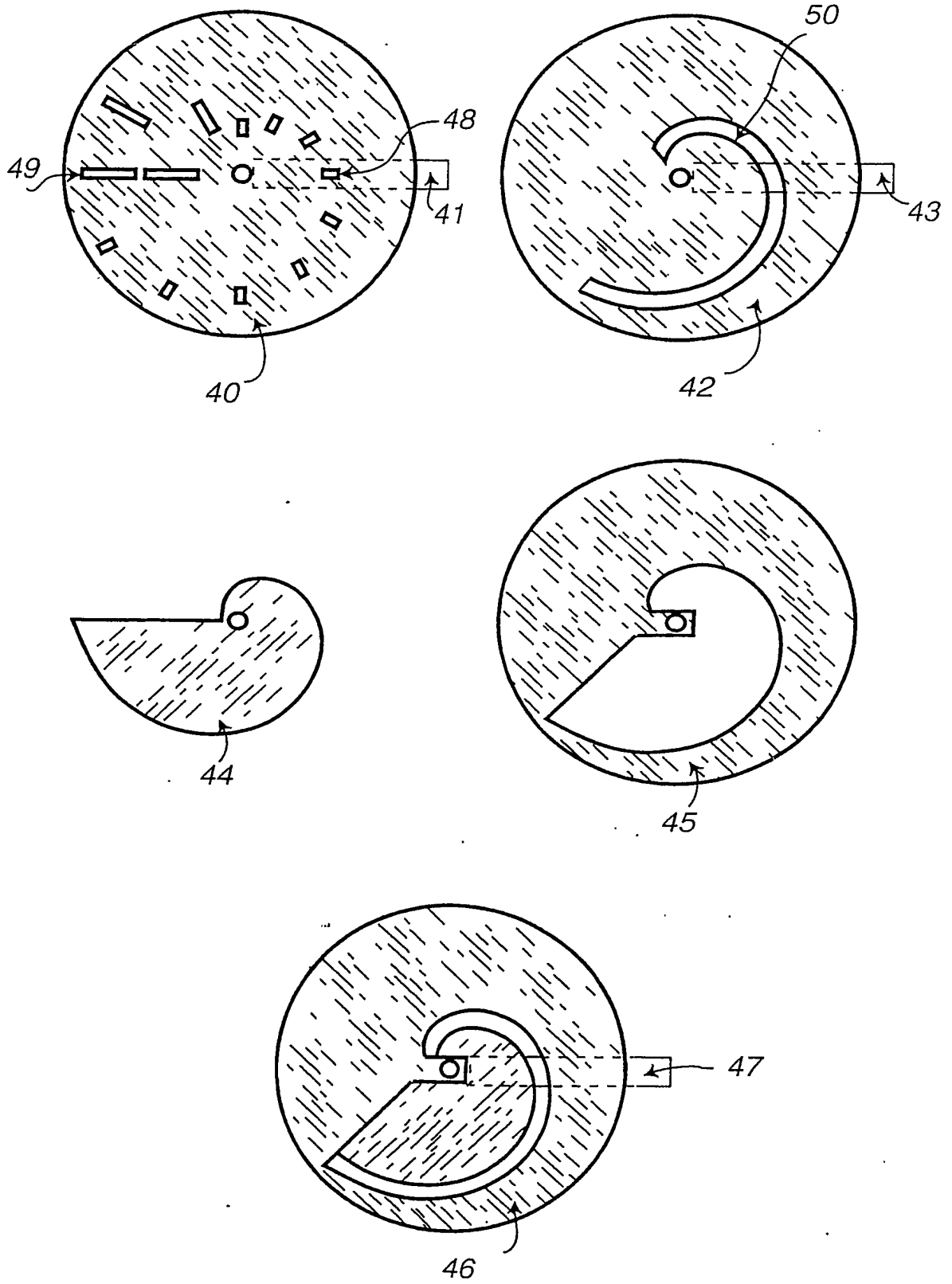


Figure 3

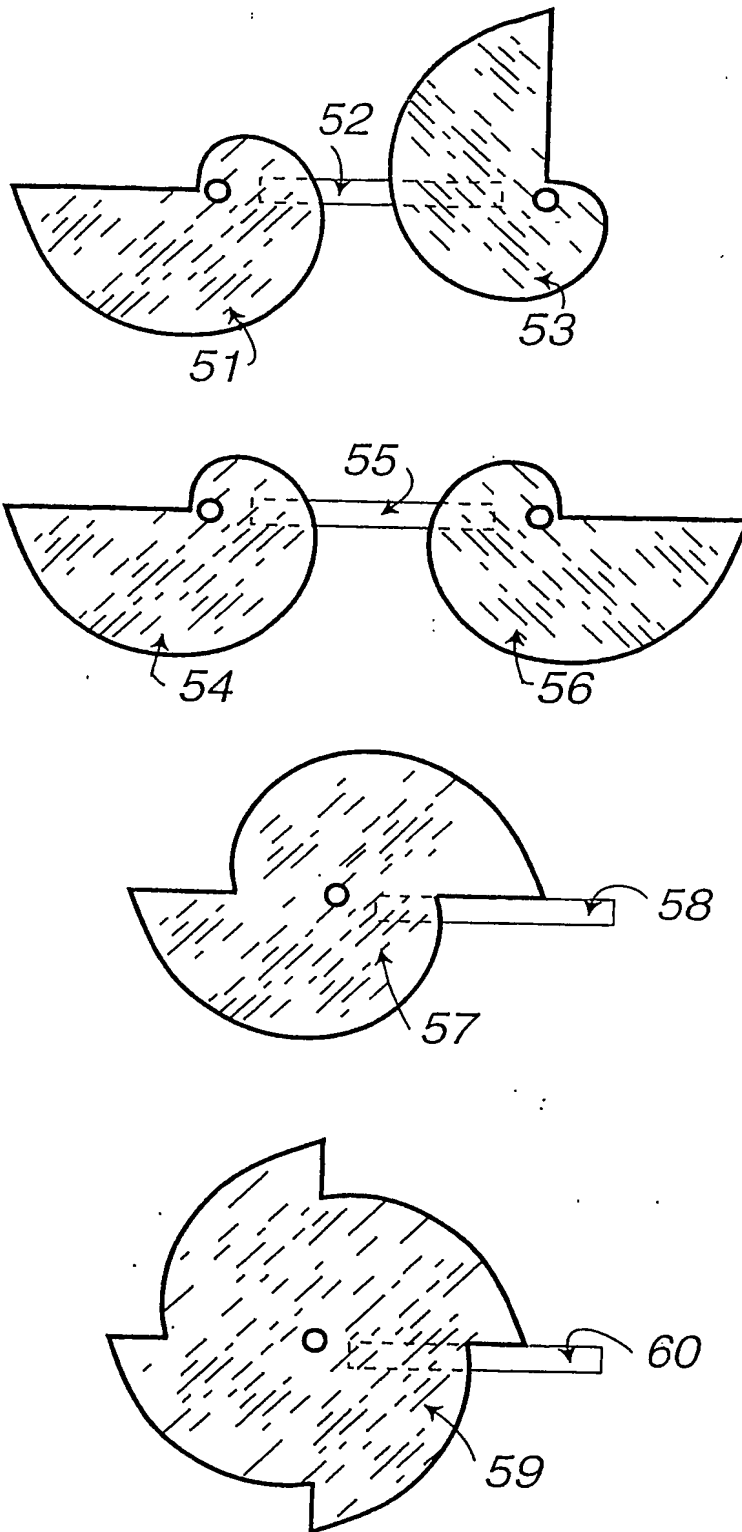


Figure 4